REVIEW

Spectrum of enteropathogens detected by the FilmArray GI Panel in a multicentre study of community-acquired gastroenteritis

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Abstract

The European, multicentre, quarterly point-prevalence study of community-acquired diarrhoea (EUCODI) analysed stool samples received at ten participating clinical microbiology laboratories (Austria, Finland, France, Germany, Greece, Ireland, Italy, Portugal, Romania, and the UK) in 2014. On four specified days, each local laboratory submitted samples from ≤20 consecutive patients to the Austrian Study Centre for further testing with the FilmArray GI Panel (BioFire Diagnostics, Salt Lake City, UT, USA). Of the 709 samples from as many patients received, 325 (45.8%) tested negative, 268 (37.8%) yielded only one organism, and 116 (16.4%) yielded multiple organisms. Positivity rates ranged from 41% (30 of 73 samples) in France to 74% (59 of 80 samples) in Romania. With the exception of *Entamoeba histolytica* and *Vibrio cholerae*, all of the 22 targeted pathogens were detected at least once. Enteropathogenic *Escherichia coli*, *Campylobacter* species, toxigenic *Clostridium difficile*, enteroaggregative *E. coli*, norovirus and enterotoxigenic *E. coli* were the six most commonly detected pathogens. When tested according to local protocols, seven of 128 positive samples (5.5%) yielded multiple organisms. Overall, the FilmArray GI Panel detected at least one organism in 54.2% (384/709) of the samples, as compared with 18.1% (128/709) when testing was performed with conventional techniques locally. This underlines the considerable potential of multiplex PCR to improve routine stool diagnostics in community-acquired diarrhoea. Classic culture methods directed at the isolation of specific pathogens are increasingly becoming second-line tools, being deployed when rapid molecular tests give positive results. This optimizes the yield from stool examinations and dramatically improves the timeliness of diagnosis.

Keywords: Community-acquired, gastroenteritis, gastrointestinal pathogens, multiplex PCR detection, stool
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Introduction

Data regarding the enteric pathogens responsible for community-acquired diarrhoeal illness in Europe are scarce, and most published studies report single-country data [1–6]. Even when diagnostic efforts are pursued aggressively, an agent cannot be identified for almost half of diarrhoeal cases if conventional methods, such as culture, enzyme immunoassay, or microscopy, are relied on for the detection of enteropathogens, either because the pathogen is not detected or because the aetiology is non-infectious [7–11]. Numerous publications have already shown the added value of molecular multiplex detection of enteropathogens in comparison with conventional methods [12–14]. We report the first European, multicentre, cross-sectional quarterly point-prevalence study of community-acquired diarrhoea

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(EUCODI) to determine the spectrum of possible pathogens in acute community-acquired gastroenteritis using both conventional laboratory techniques and a commercially available multiplex PCR-based system, in order to obtain insights into the aetiology of enteropathogens in Europe.

Materials and methods

Samples

Laboratories (one from each of ten European countries (Fig. 1)) were recruited to collect ≤20 stool samples each, on four days in 2014 (15 January, 16 April, 16 July, and 15 October), reflecting seasonal variation in disease incidence. Countries were chosen to reflect a wide geographical and socio-economic range. Laboratories in each country were identified pragmatically on the basis of established links and willingness to participate. All unformed faecal samples from outpatients or inpatients (within 48 h of admission) of all ages admitted with community-acquired acute gastroenteritis were eligible for inclusion. Second or subsequent samples from identical patients, solid samples and samples with clinical histories suggesting non-infectious causes of diarrhoea were excluded.

Microbiology

Samples were routinely tested at local laboratories according to the individual laboratories' standard operating procedures. Thereafter, each local laboratory transferred 500-µL (or gram-equivalent) aliquots into 2 mL of modified Cary–Blair medium (LBM FecalSwabs; Copan Diagnostics, Murieta, CA, USA) for transport via courier service to the central study laboratory in Vienna, Austria. At the central study laboratory, all stool samples were tested with a development version of a commercially available multiplex PCR system, the FilmArray GI Panel (BioFire

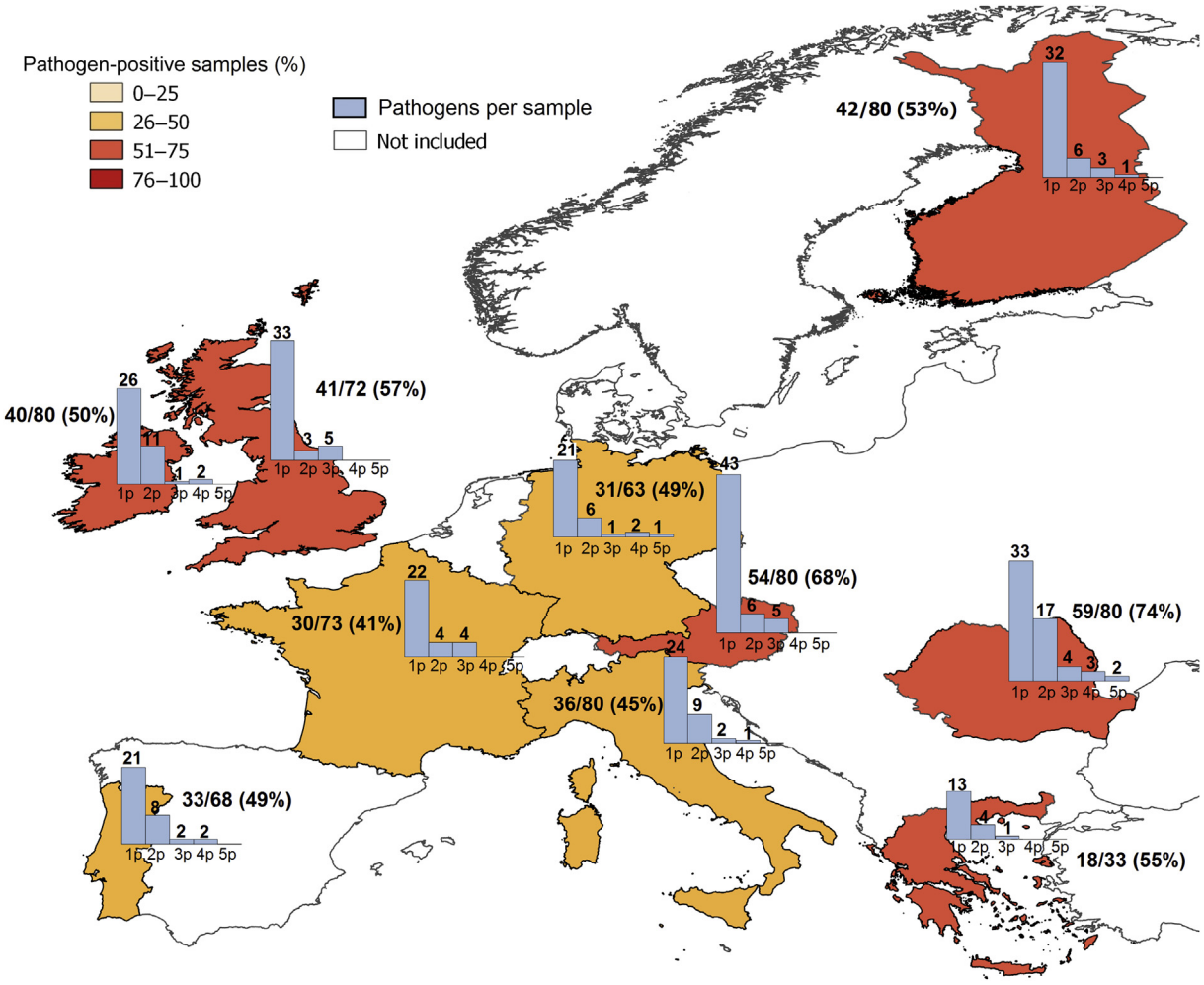

FIG. 1. Proportion of positive samples and pathogens per sample by country (Austria, Finland, France, Germany, Greece, Ireland, Italy, Portugal, Romania, and the UK; overall positivity rate = 384/709; 54.2%).

TABLE 1. Patient characteristics of samples received by round and overall

Characteristic	Round 1	2	3	4	Total	p
Female, *n* (%)	81 (51.6)	89 (49.4)	95 (49.0)	101 (56.7)	366 (51.6)	0.431
Median age (IQR)	36 (51)	37 (43)	46 (41)	47 (44)	41 (43)	0.954
<5 years, *n* (%)	26 (16.6)	27 (15.0)	22 (11.3)	33 (18.5)	108 (15.2)	0.261
5–59 years, *n* (%)	91 (58.0)	102 (56.7)	107 (55.2)	88 (49.4)	388 (54.7)	0.398
≥60 years, *n* (%)	40 (25.5)	51 (28.3)	65 (33.5)	57 (32.0)	213 (30.0)	0.355
Healthcare provider, *n* (%)						
Community based	104 (66.2)	135 (75.0)	130 (67.0)	102 (57.3)	471 (66.4)	0.006
Outpatient hospital based	3 (1.91)	6 (3.3)	25 (12.9)	38 (21.4)	72 (10.2)	<0.001
Inpatient hospital based	50 (31.9)	39 (21.7)	39 (20.1)	38 (21.4)	166 (23.4)	0.043
Antibiotic treatment, *n* (%)	11 (7.0)	21 (11.7)	12 (6.2)	18 (10.1)	62 (8.7)	0.21
Median days from sampling to analysis (IQR)	8 (3)	13 (7)	12 (7)	11 (7)	11 (7)	<0.001
Positive samples, *n* (%)	81 (51.6)	98 (54.4)	102 (52.6)	103 (57.9)	384 (54.2)	0.579
Total, *N*	157	180	194	178	709	

IQR, interquartile range.

Diagnostics, Salt Lake City, UT, USA). This panel allows simultaneous detection of 22 common diarrhoeal agents, including bacteria, viruses, and protozoa (with a total of 1 h of run time and approximately 2 min of preparation time), as follows: bacteria—*Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter upsaliensis*, toxigenic *Clostridium difficile*, *Plesiomonas shigelloides*, *Salmonella*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Vibrio cholerae*, *Yersinia enterocolitica*, enterotoxigenic *Escherichia coli* (ETEC) (*lt*/*st*), enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (*stx1*/*stx2*, including *E. coli* O157), *Shigella*/enteroinvasive *E. coli*, and enteroaggregative *E. coli* (EAEC); viruses—adenovirus F40/41, astrovirus, norovirus GI/GII, rotavirus A, and sapovirus (genogroups I, II, IV, and V); and protozoa—*Cryptosporidium*, *Cyclospora cayetanensis*, *Entamoeba histolytica*, and *Giardia lamblia*.

Demographic data

Standardized forms for each stool sample detailed the following demographic data: age, sex, healthcare facility requesting testing (outpatient hospital-based provider vs. inpatient hospital-based provider vs. community-based provider), and receipt of antimicrobial therapy (if known).

Statistical analysis

In univariate analyses, categorical variables were tested with the chi-squared test, and one-way analysis of variance was used for continuous variables. Statistical analyses were performed with STATA version 13 (StataCorp, College Station, TX, USA). Figures were created with Microsoft Excel 2010 and QGIS version 2.0.1-Dufour.

Ethical approval

The ethics commission of the city of Vienna determined (according to regulation EK 13-151-VK_NZ) that the study did not require formal ethical review.

Results

A total of 709 samples were received from the ten participating countries: Austria, 80 samples (11.3% of all samples); Finland, 80 (11.3%); France, 73 (10.3%); Germany, 63 (8.9%); Greece, 33 (4.7%); Ireland, 80 (11.3%); Italy, 80 (11.3%); Portugal, 68 (9.6%); Romania, 80 (11.3%); and the UK, 72 (10.2%). The proportion of samples received per country did not vary significantly by round (p 0.752).

Table 1 shows patient characteristics for the samples received; of 709 patients providing anonymized samples, the median age was 41 years (inter-quartile range (IQR) 43 years), 366 (51.6%) were female and 388 (54.7%) were aged 5–59 years. The majority of samples were from community-based healthcare providers (471 patients; 66.4%). A minority of patients (62; 8.7%) had received antibiotic treatment. Patients providing samples did not differ significantly in sex distribution, age, or receipt of antibiotic therapy, or by round. The settings of healthcare providers sending samples did differ significantly between rounds, especially 'outpatient hospital based' ($p < 0.001$).

Results of the central study laboratory using the FilmArray GI Panel

A total of 745 runs were needed to analyse all 709 samples on the FilmArray GI Panel (data not shown). Failed attempts ($n = 36$; 4.8%) were attributable to either software errors (ten runs/36 fails; 27.8%) or loss of vacuum pressure in the pouches (26 runs/36 fails; 72.2%). The median number of days between sampling and testing at the central study laboratory was 11 (IQR 7 days).

The overall positivity rate was 384 positive samples among 709 specimens (54.2%), with similar rates in each round (Table 1). Among the 709 samples that were screened, in 187 a single bacterium was detected (26.4%), in 62 a single virus

(8.7%), and in 19 a single protozoan (2.7%); 116 (16.4%) contained multiple pathogens. Fig. 1 shows the positivity rate by participating country. Positivity rates ranged from 41% (30 of 73 samples) in France to 74% (59 of 80 samples) in Romania.

With the exception of *Entamoeba histolytica* and *V. cholerae*, all of the 22 targeted pathogens were detected at least once. A total of 555 potential pathogens were detected in the 384 positive samples; the overall frequency distributions and those among multiple pathogen samples are shown in Fig. 2. EPEC, *Campylobacter*, toxigenic *C. difficile*, EAEC, norovirus and ETEC were the most commonly detected pathogens. Detection of enteric pathogens varied by age group. Fig. 3 shows, in particular, that *C. difficile* and EPEC occurred more frequently in the >60-year age group, whereas norovirus and EPEC were more prevalent in the <5-year age group. Pathogens with significant prevalence differences by age group were as follows: *C. difficile* (p 0.010), *Salmonella* (p 0.005), adenovirus (p 0.007), norovirus ($p < 0.001$), rotavirus ($p < 0.001$), and sapovirus ($p < 0.001$).

The frequency of bacteria, viruses and protozoa found by sampling round (total pathogens = 555) varied by season: 15 January—73 bacterial, 36 viral and 12 protozoan agents; 16 April—93 bacterial, 32 viral and six protozoan agents; 16 July—127 bacterial, 22 viral and four protozoan agents; and 15 October—115 bacterial, 26 viral and 9 protozoan agents. Bacteria were the most commonly occurring pathogens (n = 408), and were more frequently present in summer (July), whereas viruses and protozoa occurred more frequently in winter (January). However, none of these differences was found to be statistically significant.

In 116 of the 709 samples (16.4%; 30.2% of 384 positive samples), multiple pathogens were detected. Patients with multiple pathogens were more likely to be < 5 years of age (p 0.008) and be hospital outpatients (p 0.001) than those with a negative sample or with a single-pathogen sample (Table 2). On checking for statistical differences between single infection and no pathogen detected in age groups and inpatients and outpatients, the only significant result was that those with a single infection were less likely to be taking antibiotics than those with no infection (p 0.038) (artefact resulting from the small numbers taking antibiotics). On comparison of those who were infected (any number) with those who were not, the former were more likely to be aged <5 years (p 0.001), were more likely to be community based (p 0.008), and were less likely to be taking antibiotics (p 0.025).

The most commonly occurring co-infection was *Campylobacter* with EPEC (eight samples; 6.9% of samples with multiple infections and 2.1% of positive samples), followed by triple infection with EAEC, EPEC, and ETEC (seven samples; 6.0% of multiple infections and 1.8% of positive samples) (Table 3). EPEC or EAEC was present in 98 of 116 (84%) samples with multiple pathogens detected. Indeed, 85% of EAEC-positive samples and 54% of EPEC-positive samples contained other pathogens (Fig. 2). As seen in Fig. 1, samples from Romania most frequently had multiple pathogens, including two samples

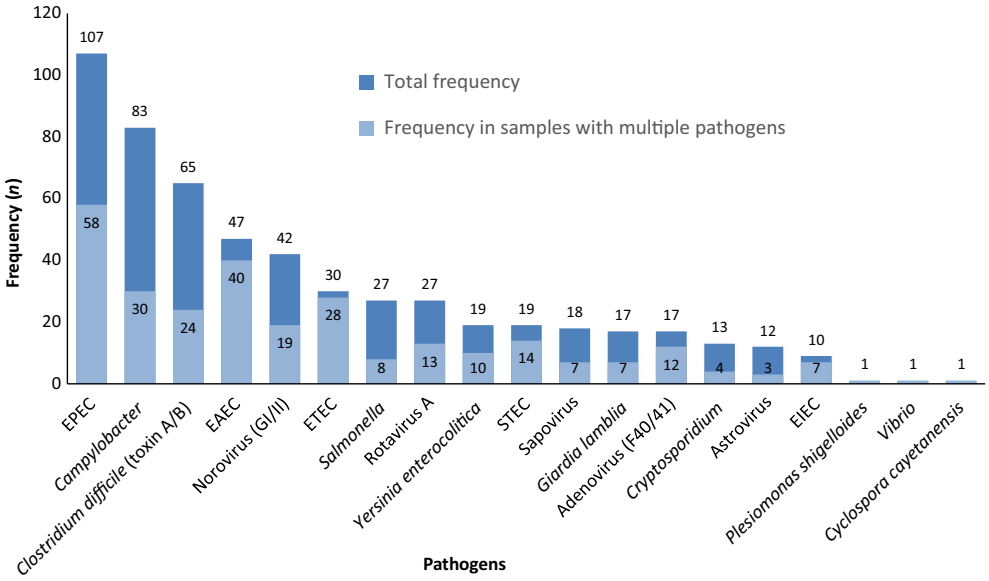

FIG. 2. Overall frequency distribution of pathogens detected and frequency distribution of pathogens that were detected in a sample with multiple pathogens detected (total pathogens = 555). EAEC, enteroaggregative *Escherichia coli*; EIEC, enteroinvasive *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*; ETEC, enterotoxigenic *Escherichia coli*; STEC, Shiga toxin-producing *Escherichia coli*.

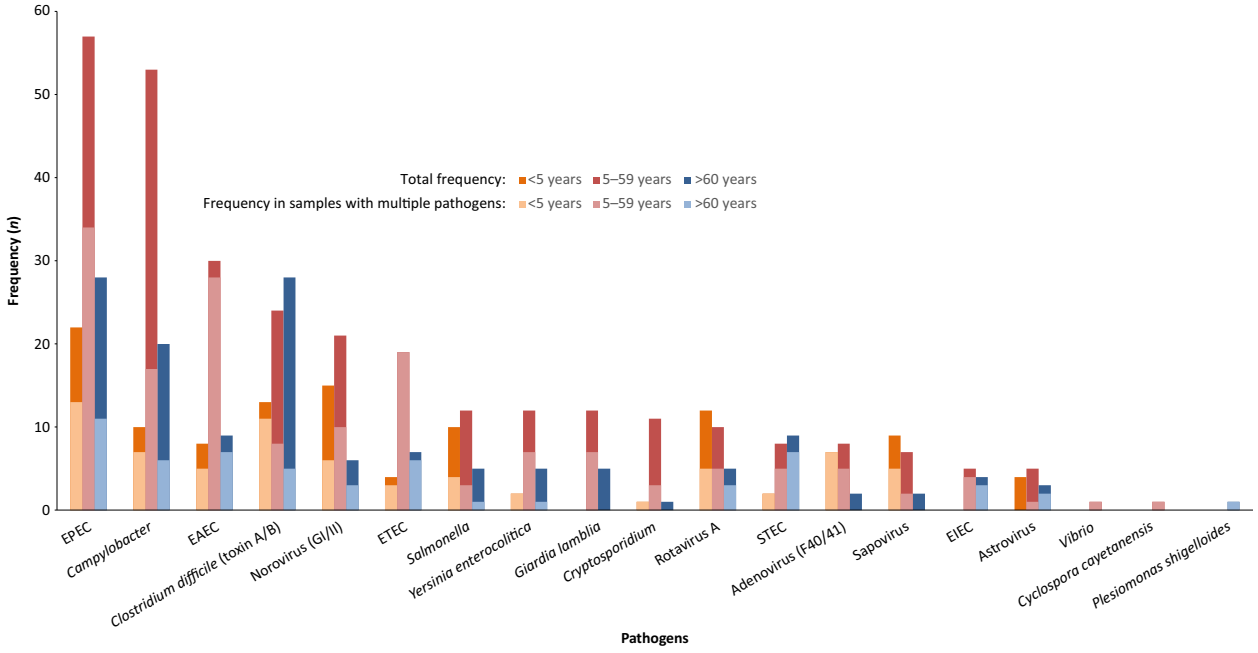

FIG. 3. Frequency distribution of pathogens detected and frequency distribution of pathogens that were detected in a sample with multiple pathogens detected by age group (<5 years, 5–59 years, and >60 years) (total pathogens = 555). EAEC, enteroaggregative *Escherichia coli*; EIEC, enteroinvasive *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*; ETEC, enterotoxigenic *Escherichia coli*; STEC, Shiga toxin-producing *Escherichia coli*.

TABLE 2. Patient demographics comparing samples with multiple pathogens and those without (*N* = 709)

Characteristic	Multiple pathogens (*n* = 116)	Single pathogen or not detected (*n* = 593)	p
Female, *n* (%)	61 (52.6)	305 (51.4)	0.82
Median age (IQR)	32 (47)	44 (43)	0.838
<5 years, *n* (%)	27 (23.3)	81 (13.7)	0.008
5–59 years, *n* (%)	66 (56.9)	322 (54.3)	0.607
≥60 years, *n* (%)	23 (19.8)	190 (32.0)	0.009
Healthcare provider, *n* (%)			
Community based	72 (62.1)	399 (67.3)	0.277
Outpatient hospital based	22 (19.0)	50 (8.4)	0.001
Inpatient hospital based	22 (19.0)	144 (24.3)	0.216
Antibiotic treatment, *n* (%)	11 (9.5)	51 (8.6)	0.758

IQR, interquartile range.

each containing five pathogens. One contained *Campylobacter*, *C. difficile* (toxin A/B), norovirus (GI/II), rotavirus A, and sapovirus; the other contained *C. difficile* (toxin A/B), EAEC, EPEC, norovirus (GI/II), and sapovirus. Both of these samples were from hospital outpatients. A further sample from Germany contained five pathogens: *C. difficile* (toxin A/B), EAEC, ETEC, norovirus (GI/II), and rotavirus A.

The positive *Vibrio* result was in a sample from Austria, and was due to *V. parahaemolyticus*; routinely seeded blood agar plates had grown abundant oxidase-positive colonies (species identification confirmed by the Austrian National *Vibrio* Reference Laboratory).

With the exception of Romania (where toxigenic *C. difficile* was by far the most common pathogen; 13 of 25 Romanian *C. difficile* cases (52%) were aged >60 years), in all cases the most commonly occurring pathogen was either EPEC or *Campylobacter* (Fig. 4). EPEC was among the top three most frequent pathogens for all participating countries. *Campylobacter* was among the top five pathogens for all countries except France, Germany, and Romania (where it was ninth, eighth, and sixth, respectively). Toxigenic *C. difficile* was among the top three pathogens for all countries except Austria, Ireland, Italy, and the UK. Norovirus, except in Romania and the UK, was always among the top six pathogens. Sapovirus was in the top five pathogens in Ireland and Italy, and in the UK, where sapovirus occurred more frequently than norovirus. Additionally, the UK had the highest frequency of *Giardia lamblia*, it being the fourth most common pathogen. Ireland had a high frequency of adenovirus, which was not noted elsewhere.

Results of the local laboratories using conventional laboratory techniques

Of the 709 samples, 581 (81.9%) were negative when tested according to the local laboratory protocols. *Campylobacter* was the most common pathogen (56 samples; 7.9%), followed by *Salmonella*, toxigenic *C. difficile*, rotavirus, and norovirus (20 (2.8%), 17 (2.4%), 14 (2.0%), and ten (1.4%), respectively). EPEC, the most common pathogen identified on the FilmArray

TABLE 3. Frequency of multiple pathogen combinations (displaying those with a frequency of ≥2) and proportion of total samples with multiple pathogens detected (n = 16 multiples/384 positive samples)

Pathogens	Frequency of samples	Proportion of samples with multiple pathogens	Proportion of positive samples
Campylobacter species + EPEC	8	6.9	2.1
EAEC + EPEC + ETEC	7	6.0	1.8
EPEC + norovirus (GI/II)	4	3.5	1.0
EAEC + EIEC	3	2.6	0.8
EAEC + EPEC	3	2.6	0.8
EPEC + *Giardia lamblia*	3	2.6	0.8
Adenovirus (F40/41) + norovirus (GI/II)	2	1.7	0.5
Clostridium difficile (toxin A/B) + adenovirus (F40/41) + sapovirus	2	1.7	0.5
C. difficile (toxin A/B) + EPEC	2	1.7	0.5
C. difficile (toxin A/B) + ETEC	2	1.7	0.5
C. difficile (toxin A/B) + STEC	2	1.7	0.5
Campylobacter + *C. difficile* (toxin A/B)	2	1.7	0.5
Campylobacter + *C. difficile* (toxin A/B) + EPEC	2	1.7	0.5
Campylobacter + EAEC + EPEC	2	1.7	0.5
Campylobacter + STEC	2	1.7	0.5
EAEC + EPEC + ETEC + EIEC	2	1.7	0.5
EAEC + norovirus (GI/II)	2	1.7	0.5
EPEC + *Cryptosporidium*	2	1.7	0.5
EPEC + rotavirus A	2	1.7	0.5
ETEC + rotavirus A	2	1.7	0.5
STEC + norovirus (GI/II)	2	1.7	0.5
STEC + rotavirus A	2	1.7	0.5
Salmonella + EAEC	2	1.7	0.5
Salmonella + EPEC	2	1.7	0.5
Subtotal	64	55.2	17.7
Total multiple pathogens detected	116	100.0	30.2

EAEC, enteroaggregative *Escherichia coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; STEC, Shiga toxin-producing *E. coli*.

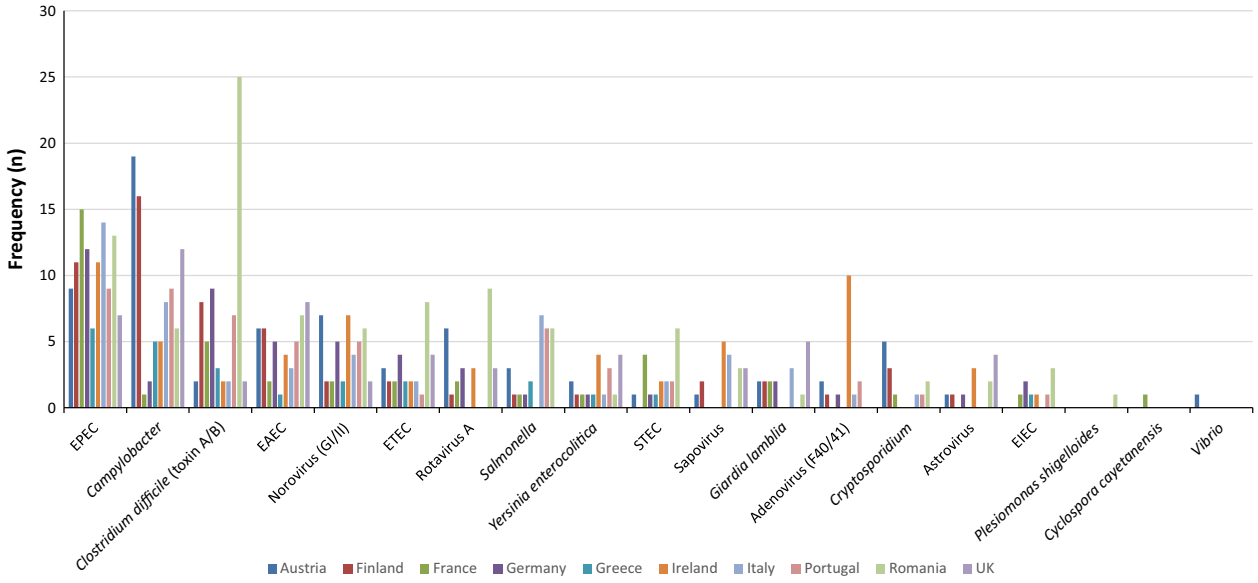

FIG. 4. Frequency distribution of pathogens detected by country (total pathogens = 555). EAEC, enteroaggregative *Escherichia coli*; EIEC, enteroinvasive *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*; ETEC, enterotoxigenic *Escherichia coli*; STEC, Shiga toxin-producing *Escherichia coli*.

GI Panel, was detected in only three samples (0.4%), and EAEC was not detected in any. Only Germany, Italy and Romania tested for EPEC, and none of the participating laboratories tested for EAEC. Only seven samples contained two pathogens (1.0%), with no combinations occurring more than once; four of the seven contained a diarrhoeagenic *E. coli*. Fig. 5 shows the results of the two diagnostic approaches for comparison.

Discussion

The results of the EUCODI study impressively underline the wide spectrum of possible enteric pathogens in patients with community-acquired gastroenteritis: with the exception of *Entamoeba histolytica* and *V. cholerae*, all of the 22 targeted

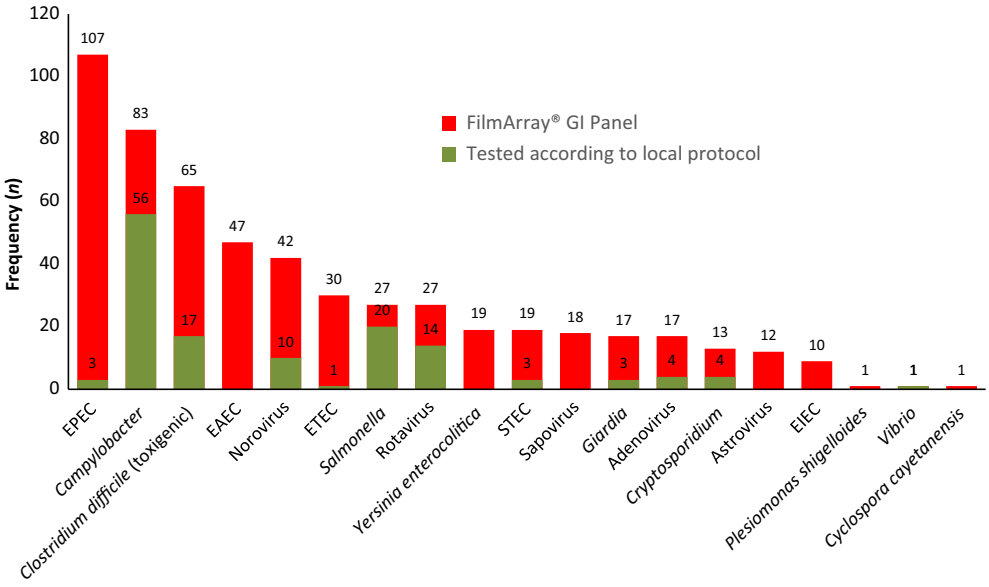

FIG. 5. Frequency distribution of pathogens detected in 709 stool samples by diagnostic approach employed: local laboratory protocol vs. FilmArray GI Panel. EAEC, enteroaggregative *Escherichia coli*; EIEC, enteroinvasive *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*; ETEC, enterotoxigenic *Escherichia coli*; STEC, Shiga toxin-producing *Escherichia coli*.

potential pathogens were detected at least once. This underscores the importance of using a comprehensive test spectrum for the work-up of samples from patients with community-acquired gastroenteritis. In our European study, the FilmArray GI Panel detected at least one organism in 54% of the samples, whereas the local laboratory protocols detected at least one organism in 18.1% of the samples. Data from the manufacturer's clinical trials in the USA had yielded a nearly identical positivity rate: possible pathogens were detected in 832 of 1556 patients (53.5%) [13]. Among 230 samples collected prospectively in 2013–2014 at the Mayo Clinic in Rochester MN, 76 (33.0%) were positive for one or more gastrointestinal (GI) pathogens by FilmArray GI Panel testing vs. 8.3% by routine testing [14]. In Austria, stool specimens submitted to a private laboratory and to the laboratory of a university hospital for routine testing (e.g. culture, antigen testing, microscopy, and individual real-time PCR) in 2014 yielded positive results for one or more GI pathogens in only 8.5% and 2.7% of samples, respectively (unpublished data).

In the above-mentioned prospective study at the Mayo Clinic in Rochester MN, the FilmArray GI Panel identified mixed infections in 21.1% of positive prospective samples, and routine methods detected them in 8.3% [14]. In our European study, the FilmArray GI Panel detected multiple organisms in 16.4% of samples (representing 30% of positive samples), whereas routine methods as applied by the participating laboratories detected them in only 1% of samples. Our findings corroborate the suggestion of Khare *et al.* that the presence of multiple pathogens in diarrhoeal stool samples is underestimated by current routine tests [14].

EPEC or EAEC was present in 98 of 116 (84%) of our EUCODI samples with multiple organism detection, which raises the question of the clinical relevance of these putative pathogens, or challenges the validity of the pathogen definitions used. We believe that all of these EPEC and EAEC results could require further investigation (e.g. serotyping of isolates). EPEC and EAEC are not routinely tested for in most laboratories, including the laboratories participating in EUCODI. In our study, EPEC, *Campylobacter*, toxigenic *C. difficile*, EAEC, norovirus and ETEC were the six most commonly detected pathogens. Also at Mayo, EPEC topped the list, with EAEC again being the fourth most commonly diagnosed potential agent (EPEC, toxigenic *C. difficile*, sapovirus, EAEC, norovirus, *Campylobacter*, and ETEC) [14]. The high rate of EPEC and EAEC detection in the stools of patients—but also in controls—has previously been reported by others [15,16].

The EUCODI study confirms that rotavirus and *Salmonella* are no longer the leading viral and bacterial GI pathogens (respectively) in Europe. Epidemiological studies have repeatedly reported changes in the prevalence rates of various causative agents in some European countries [5,6,8–10]. In Europe, the inclusion of a childhood vaccine for rotavirus in national immunization programmes (as recommended by the WHO since 2009) and the legal obligation for member states to take effective measures to control *Salmonella* in laying hen flocks

[17,18] have significantly changed the pathogen spectrum of community-acquired gastroenteritis during the last few years.

Presently, norovirus has been identified as the leading cause of medically attended acute community-acquired gastroenteritis in Europe; our finding is in accordance with the situation in the USA [19]. However, both our data and the findings from the Mayo study [14] also underscore that rotavirus and sapovirus should also be part of the routine test spectrum for community-acquired diarrhoea. In many laboratories, ordering physicians must still specifically request such testing. Leaving EPEC—which is currently not in the diagnostic repertoire of most routine diagnostic laboratories—aside, our study suggests that *Campylobacter* and toxigenic *C. difficile* are the leading causes of community-acquired bacterial enteritis in Europe, again in accordance with the situation in the USA [14]. In our study, the high *C. difficile* prevalence in Romania does skew the frequency distribution; however, if the Romanian cases are excluded, *C. difficile* is still the fifth most frequently detected pathogen (40, 17 of which were in multiple-pathogen samples), moving it behind EAEC and norovirus in the ranking.

It is common practice at present to test for toxigenic *C. difficile* only in hospitalized patients or in those with other risk factors, such as a history of recent antibiotic use or if other testing gives negative results [1,2]. However, in the last decade, there has also been a significant increase in the detection of community-acquired *C. difficile* infection (CDI). Across Europe, 14% of CDI cases were found to be community associated in a study in 2008 by Bauer *et al.* [20]. In a study on acute gastroenteritis in general practices in Austria in 2007, *C. difficile* accounted for 18.7% of positive results, as compared with 9.3% for *Campylobacter* and 6.6% for *Salmonella* (norovirus, 36%; rotavirus, 17.3%; and adenovirus, 5.4%) [10]. Considering the importance of toxigenic *C. difficile*, we conclude that laboratories should evaluate the need for routine toxigenic *C. difficile* testing of samples from patients with community-acquired diarrhoea. In this context, we note that, in contrast to EPEC, toxigenic *C. difficile* is infrequently found in healthy adults [21,22]. Current guidelines for the diagnosis of CDI state that algorithm testing should be used, as stand-alone tests are not suitable with regard to sensitivity/specificity, especially when tests are performed in low-prevalence populations [23].

Community-acquired GI infections show a seasonal pattern. The wintertime predominance of norovirus infection is so marked that it has been called 'winter-vomiting disease'. Campylobacteriosis and salmonellosis occur more commonly during the summer [24]. Although our data show the descriptive trends in absolute numbers noted in the literature, they do not show statistically significant variation (probably because of the low sample size). However, this seasonality is insufficiently marked to warrant seasonal omission of certain targets from the test spectrum for community-acquired diarrhoea.

Some organisms may be carried asymptomatically. Detection of organisms by conventional methods or by molecular diagnostics may therefore not mean that the corresponding organisms are responsible for a patient's symptoms. The FilmArray GI Panel and molecular diagnostics in general have another inherent limitation: viral, bacterial and parasite nucleic acid may persist *in vivo*, independently of organism viability [12]. Discrepancies between the FilmArray GI Panel and other microbial identification methods may also be caused by the inability to reliably differentiate species with standard phenotypic microbial identification methods. Examples include the differentiation of *Y. enterocolitica* from other *Y. enterocolitica* group members, and the differentiation of *E. histolytica* from *Entamoeba dispar*. There is also a risk of false-negative results due to the presence of sequence variants in the gene targets of the assay, procedural errors, or inadequate numbers of organisms for amplification. In our opinion, these inherent limitations are far outweighed by the numerous advantages of an automated multiplex PCR system. The relatively high rate of failed attempts noted in our EUCODI study (4.8% of our tests had to be repeated) was most likely due to the use of developmental pouches, and was higher than that reported during the prospective clinical evaluation of the product by the manufacturer (0.6%) [13].

With the exception of toxigenic *C. difficile* and diarrhoeagenic *E. coli* targets, the chosen gene targets are proprietary. Although the published performance data substantiate the appropriateness of the chosen targets [13,14], we feel that operators of the FilmArray GI Panel should have access to such details.

Our EUCODI study has clear limitations. The first concerns the representativeness: the participating laboratories were chosen mainly because of their engagement in the ESCMID Food- and Water-borne Infections Study Group. These laboratories may not necessarily be representative of their country. Similarly, the ten countries identified do not represent all member states within the EU. Second, the number of samples was rather low (n = 709), and its statistical power should be interpreted with caution. Despite these limitations, we consider that multicentre, cross-sectional point-prevalence studies of community-acquired diarrhoea are suitable for generating valid, up-to-date knowledge concerning the spectrum of possible pathogens in acute community-acquired gastroenteritis in Europe. We feel that our results call for a more comprehensive comparative study, as this limited EUCODI study clearly shows feasibility. Such a comprehensive comparative study would allow a critical appraisal of the validity of information on communicable GI diseases provided by official surveillance

systems based on statutorily reported illnesses and results from routine laboratories, e.g. the 2014 Annual Epidemiological Report of the European Centre for Disease Prevention and Control [25] and the EU Summary Report on Zoonoses, Zoonotic Agents and Food-Borne Outbreaks 2012 [26]. The present lack of standardization of various diagnostic assays deployed in European laboratories inevitably hampers such reports, and makes any direct comparison between countries impossible. According to the EU summary report, the country-specific notification rates of reported confirmed cases of human campylobacteriosis in the EU in 2012 were highest in the Czech Republic, Slovakia, Luxembourg, and the UK (106–174 per 100 000 population), and lowest rates in Bulgaria, Latvia, Italy, Poland, and Romania (<2 per 100 000 population) [26]. Whereas in 2012 (according to the EU summary report) the ratios of confirmed cases of campylobacteriosis in the UK and Romania were 273 : 1, and 250 : 1, respectively (according to the annual European Centre for Disease Prevention and Control epidemiological report) [25], our EUCODI study yielded a ratio of 2 : 1 for *Campylobacter*-positive specimens (UK, 12 of 72 samples positive for *Campylobacter*; Romania, six of 80 samples positive for *Campylobacter*) in 2014.

We conclude that multiplex screening can optimize the yield from stool examinations, can dramatically improve the timeliness of diagnosis, and can facilitate comparison of results among different countries.

Transparency declaration

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